p-Hydroxybenzyl Alcohol Attenuates Learning Deficits in the Inhibitory Avoidance Task: Involvement of Serotonergic and Dopaminergic Systems

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Abstract

***p*-Hydroxybenzyl alcohol (HBA), an aglycone of gastrodin, is an active ingredient of Gastrodia elata B_{LUME}. In this study, we investigated the action of HBA on acquisition of an inhibitory avoidance response in rats and used piracetam as a positive control.**

The results indicated that scopolamine, a cholinergic receptor antagonist, injected before training impaired retention. HBA did not attenuate the scopolamine-induced impairment, but piracetam did. *p*-Chloroamphetamine, a serotonin releaser, injected before training impaired retention. HBA at 5 mg/kg and piracetam at 100 mg/kg could counteract the *p*-chloroamphetamine-induced deficit. Apomorphine, a dopaminergic receptor agonist, also impaired retention. HBA at 5 mg/kg and piracetam at 300 mg/kg could ameliorate the apomorphine-induced amnesia.

The above results indicated that HBA, different from piracetam, can attenuate impairments induced by *p*-chloroamphetamine and apomorphine, but had no effect on impairment induced by scopolamine in an inhibitory avoidance task in rats. Such findings suggest that HBA may act through suppressing dopaminergic and serotonergic activities and thus improves learning.

Key Words: *p*-hydroxybenzyl alcohol, piracetam, learning acquisition, inhibitory avoidance

Introduction

p-Hydroxybenzyl alcohol (HBA) belongs to a phenolic compound and is prepared from phenol and formaldehyde by hydroxymethylation. Early studies have pointed out that HBA is the genin of gastrodin and the active ingredient of the rhizome of *Gastrodia elata* B_{LUME} (Orchidaceae) (abbreviated as GE) (1). GE, gastrodin and HBA have been shown to possess sedative, anticonvulsive and analgesic effects (2). Recent studies have shown that GE improved D-galactose-induced impairments in the step-down inhibitory avoidance task and memory deficits in senescent mice (3-4). In our previous studies, gastrodin and HBA were separated through the butanol and ethyl acetate fractions of methanol extract of GE respectively, and attenuated the scopolamine-induced deficit in an inhibitory avoidance task after chronic administration to rats (5). The mechanism of gastrodin has been reported to be due to its decomposition into HBA in the central nervous system (5-6). However, the effect of acute administration of HBA on acquisition has not been reported.

Learning generates an experience-dependent and long-lasting modification of the nervous system, which creates enduring memory representations. It involves the activation, by neurotransmitters such as acetylcholine, dopamine and serotonin, of receptor-linked enzymes responsible for synthesis of intercellular messages (7-8). A large number of studies have shown that the cholinergic system plays an important role in learning and memory (9). Scopolamine (SCOP), a cholinergic muscarinic antagonist, decreases cholinergic activity and impairs learning and memory in rodents, especially the acquisition process (10-11). The serotonergic system

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has also been implicated in learning but the decrease in serotonergic activity facilitates acquisition (12). *p*-Chloroamphetamine (PCA), a serotonergic releaser, increases serotonergic activity and impairs acquisition performance (13). Alike to the serotonergic system, the dopaminergic system is also involved in learning and the decrease in dopaminergic activity facilitates acquisition performance (14). Apomorphine (APO), a dopamine receptor agonist, at a high dose increases dopaminergic activity and impairs acquisition in rodents (15).

In the present study, we investigated the effects of acute administration of HBA on SCOP-induced, PCA-induced and APO-induced impairment in an inhibitory avoidance task. We also used the nootropic piracetam as a positive control, because it has been shown to attenuate the effects of various amnestic treatments.

Materials and Methods

Animals

Male Sprague-Dawley rats, weighing 200-250 grams, were housed in groups of six with free access to food and water in a regulated environment (23±1°C). A 12 hr light-dark cycle (light on: 8:00 to 20:00) was maintained. The number of subjects in each group was 12 to 18 and was depicted in the figure.

Drugs

p-Hydroxybenzyl alcohol (HBA), piracetam, scopolamine hydrobromide (SCOP), and *p*-chloroamphetamine hydrochloride (PCA) were purchased from Sigma Chemical Co. and dissolved in 0.9% normal saline immediately before use. Apomorphine (APO) was also purchased from Sigma Chemical Co. and dissolved immediately before use in 0.9% normal saline containing 1 mg/ml ascorbic acid to prevent oxidation.

Behavioral Task

Memory processes could be divided three stages : acquisition, consolidation and retrieval. The step-through inhibitory avoidance task was used to measure the above three stages depending on the drug-treated period. The apparatus consisted of two compartments having a steel-rod grid floor (36 parallel steel rods, 0.3 cm in diameter set 1.5 cm apart). One of the compartments (48×20×30 cm) was equipped with a 20W lamp located centrally at a height of 30 cm, and the other was dark compartment of same size, connected through a guillotine door (5×5 cm). The dark room was used during the experimental sessions that were conducted between 09:00 and 17:00 hours.

Procedure in Inhibitory Avoidance Task

During the training trial, the guillotine door connecting the light and the dark compartment was kept closed. Each rat was placed in the light compartment facing against the guillotine door. The door was then opened and simultaneously the time (step-through latency, STL) for the rat to enter the dark compartment was measured with a stopwatch. Once the rat entered the dark compartment, the door was closed. An inescapable scrambled footshock (1.0 mA for 2 s) was then delivered through the grid floor. The rat was removed from the dark compartment 5 s after administering the shock. The rat was then put back into its home cage until the retention trial, which was carried out twenty-four hours later. The rat was once again placed in the light compartment and as in the training trial, the guillotine door was opened and the step-through latency was recorded as the retention score (16). An upper cut-off limit was set at 300 s.

SCOP Pre-training Administration

In the first experiment, SCOP (1 mg/kg, i.p.) was administered 30 min before the training trial (10). HBA (1, 5 and 25 mg/kg, p.o.) was administered to SCOP-treated rats 1hr before the training trial. Piracetam (50, 100 and 300 mg/kg, p.o.) was also administered to SCOP-treated rats 1hr before the training trial. Furthermore, the dose-response effect of SCOP (0.3, 0.5 and 1 mg/kg; i.p.) was administered 30 min before the training trial. Then, HBA (1, 5 and 25 mg/kg, p.o.) was also administered to the rats treated by lower dose of SCOP.

PCA Pre-training Administration

In the second experiment, PCA (2.5 mg/kg, i.p.) was administered 30 min before the training trial (13). HBA (0.5, 1, 5 and 25 mg/kg, p.o.) was administered to PCA-treated rats 1hr before the training trial. Piracetam (50, 100 and 300 mg/kg, p.o.) was also administered to PCA-treated rats 1hr before the training trial.

APO Pre-training Administration

In the third experiment, APO (1 mg/kg, s.c.) was administered 20 minutes before the training trial (15). HBA (0.5, 1, 5 and 25 mg/kg, p.o.) was administered to APO-treated rats 1hr before the

training trial. Piracetam (50, 100 and 300 mg/kg, p.o.) was also administered to APO-treated rats 1hr before the training trial.

Response of Unshocked Rats in Inhibitory avoidance Task

To evaluate the effect of various drug combinations on motor activity in inhibitory avoidance task, the same experimental steps were followed as described in the above section with the exception that rats were not subjected to 1.0 mA inescapable footshock during the training period. Twenty-four hours later, the retention trial was carried out and the step-through latency was recorded (15). The rats were given various doses of HBA in combination with the above mentioned drugs.

Open-field Activity Measurements

The Opto-Varimex animal activity meter was used (Columbus Instruments International Co., Ltd., USA) for the measurements. The apparatus consisted of acrylic activity monitor cage (43.2×44.4×30.5 cm) which was surrounded by horizontal and vertical sensors that are non-detectable by rats. The apparatus also consisted of fifteen infrared beams spaced 2.65 cm apart, on each axis. Two infrared beams were set as sensitivity of monitors. The cage was enclosed in a ventilated cabinet. Measurements were performed between 09:00-17:00 hr.

Interruptions of photocell beams over a 5-min period were collected by a microcomputer and the activities such as grooming, scratching, digging were recorded (17). The rats were given various doses of HBA in combination with the above mentioned drugs.

Flinch-jump Test

The threshold of flinch, jump or vocalization produced by electric shock was measured by using the inhibitory avoidance task. Each rat was placed in the dark compartment of the inhibitory avoidance task apparatus and the shock intensity was manually raised stepwise from 0.5 to 1.0 mA in increments of 0.1 mA until either a flinch, jump or vocalization was observed. Duration of shock was 2 s and the intershock interval was 15 s. The point at which the rat exhibited vocalization was recorded (18). The rats were given various doses of HBA in combination with the above mentioned drugs.

Data Analysis

Because the data distribution from the inhibitory avoidance task was truncated at 300, non-parametric Kruskal-Wallis analysis followed by Mann-Whitney U-tests were used to analyze the data. The U-value was then transformed into a Z-value. The data collected during motor activity, flinch-jump test or open-field activity was analyzed with one-way analysis of variance (ANOVA) followed with Scheffe multiple range test.

Results

Experiment 1: HBA on SCOP-Induced Shortening of Retention Latency

It was observed that pre-training administration of SCOP (1 mg/kg, i.p.) remarkably decreased the retention latencies ($U=0$, $Z=4.91$, $p<0.001$). The effects of pre-training administration of HBA in various doses on SCOP-induced shortening of retention latencies were shown in Fig. 1. A Kruskal-Wallis one-way ANOVA test indicated that none of the dosage of HBA were found to alter the short latencies of the SCOP-treated rats ($H=0.71$, $p>0.05$). On the other hand, the Kruskal-Wallis test yielded a significant overall effect of piracetam on the deteriorating effect of SCOP ($H=28.00$, $p<0.001$) Further Mann-Whitney U tests indicated that piracetam at 100 and 300 mg/kg significantly counteracted the deteriorating effect of SCOP ($U=13.0$, $Z=3.12$, $p<0.01$; $U=1.0$, $Z=4.02$, $p<0.001$). The dose-response effects of SCOP injections on retention performance in rats are shown in Fig. 2.

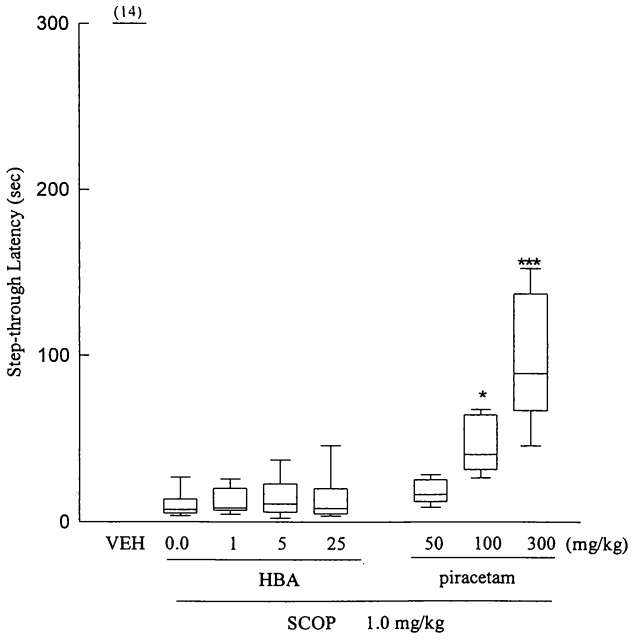

Fig. 1. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam on the SCOP-induced impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. **P<0.01, ***P<0.001 compared with SCOP group.

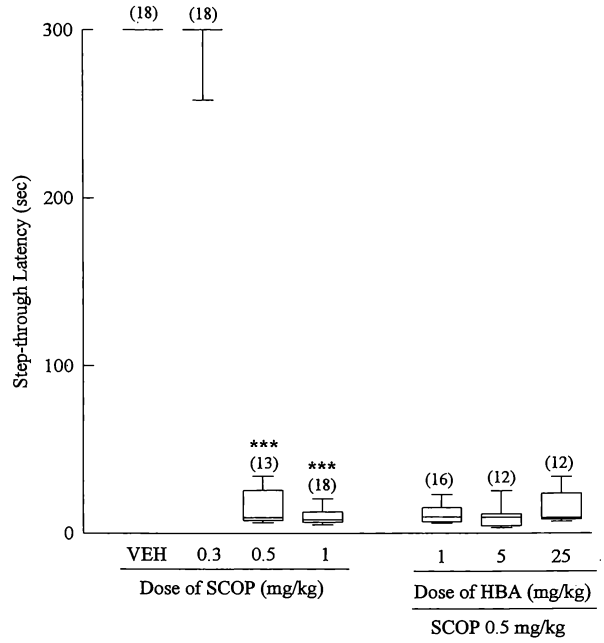

Fig. 2. Effects of *p*-hydroxybenzyl alcohol (HBA) on the SCOP-induced impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. ***P<0.001 compared with VEH group.

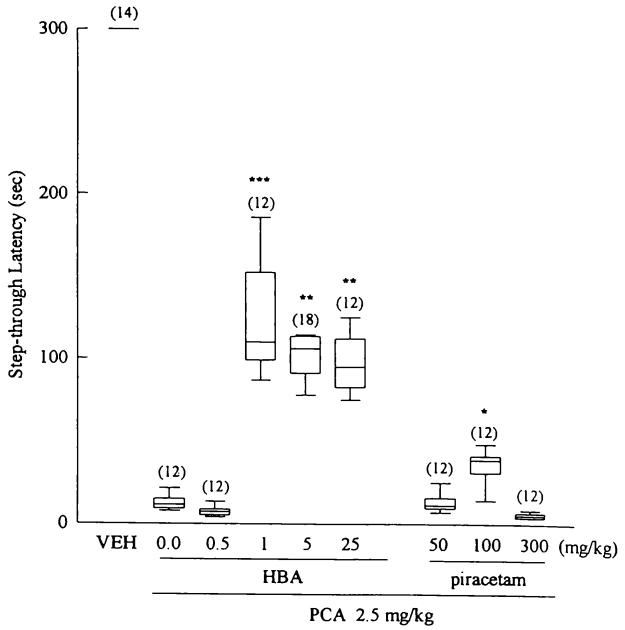

Fig. 3. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam on the PCA-induced impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. *P<0.05, **P<0.01, ***P<0.001 compared with PCA group.

SCOP at 0.5 mg/kg also markedly decreased the retention latencies (U=7, Z=4.04, p<0.001) , but not 0.3 mg/kg (U=56, Z=1.71, p>0.05). A Kruskal-Wallis one-way ANOVA test indicated that none of the dosage of HBA were found to alter the short latencies of the SCOP-treated rats (H=1.55, p>0.05).

Experiment 2: HBA on PCA-Induced Shortening of Retention Latency

It was observed that pre-training administration of PCA (2.5 mg/kg, i.p.) significantly decreased the retention latencies (U=0, Z=4.43, p<0.001). Fig. 3 shows that the PCA-induced shortening of retention latencies were rectified by pre-training administration of HBA at the doses of 1, 5 and 25 mg/kg. A Kruskal-Wallis test yielded a significant overall effect of HBA on the deteriorating effect of PCA (H=34.48, p<0.001), further Mann-Whitney U tests indicated that HBA at 1, 5 and 25 mg/kg significantly counteracted the deteriorating effect of PCA (U=0, Z=3.40, p<0.001; U=13, Z=2.70, p<0.01; U=0, Z=3.24, p<0.01). On the other hand, further Mann-Whitney U tests indicated that pre-training administration of piracetam only at 100 mg/kg significantly counteracted the PCA-induced shortening of retention latencies (U=17.0, Z=2.14, p<0.01).

Experiment 3: HBA on APO-Induced Shortening of Retention Latency

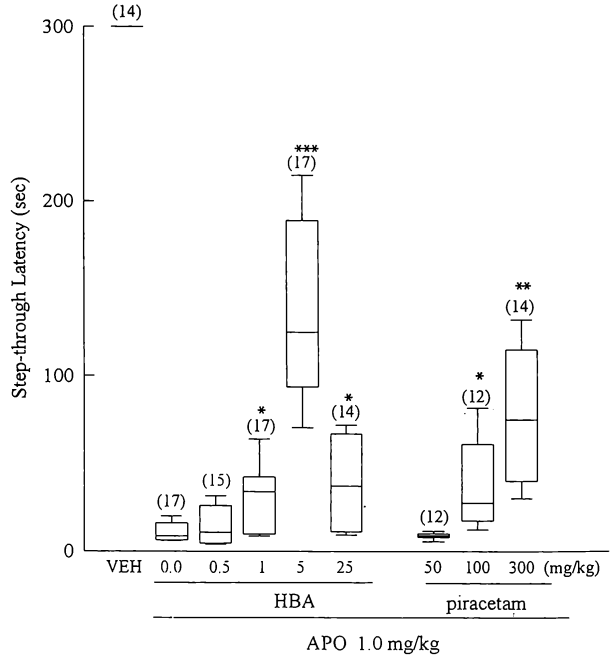

Fig. 4. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam on the APO-induced impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. *P<0.05, **P<0.01, ***P<0.001 compared with APO group.

It was observed that pre-training administration of APO (1 mg/kg, s.c.) markedly decreased the retention latencies (U=0, Z=4.70, p<0.001). Fig. 4

shows that the APO-induced shortening of retention latencies were reversed by pre-training administration of HBA at 0.5-25 mg/kg. A Kruskal-Wallis test yielded a significant overall effect of HBA on the deteriorating effect of APO ($H=32.76$, $p<0.001$). Further Mann-Whitney U tests indicated that HBA at 1, 5 and 25 mg/kg significantly counteracted the deteriorating effect of APO ($U=45.0$, $Z=2.23$, $p<0.05$; $U=1.0$, $Z=4.08$, $p<0.001$; $U=24.0$, $Z=2.54$, $p<0.05$). On the other hand, the Kruskal-Wallis test yielded a significant overall effect of piracetam on the deteriorating effect of APO ($H=27.56$, $p<0.001$). Further Mann-Whitney U tests indicated that pre-training administration of piracetam at 100 and 300 mg/kg also significantly counteracted the APO-induced shortening of retention latencies ($U=17.0$, $Z=2.98$, $p<0.05$; $U=6.0$, $Z=3.79$, $p<0.01$).

Experiment 4: HBA in Combination with Various Induced Drugs in Unshocked Rats

Pre-training administration of HBA had no effect on the step-through latencies of non-shocked rats which were given SCOP, PCA or APO in the training trial and the retention trial (Table 1-3).

Experiment 5: HBA in Combination with Various Induced Drugs in Open-Field Activity.

The movement activities of rats measured 1 hr or 25 hr after HBA administration alone were not different from those for the vehicle group (Fig. 5). The movement activities of rats measured 1 hr or 25 hr after HBA administration plus SCOP, PCA, APO were not different from those for the SCOP, PCA, APO group (Fig. 6-8).

Table 1. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam (PIR) on the step-through latency of non-shocked rats given SCOP.

Drug	Dose	Step-through Latency (sec)	
	(mg/kg)	Training trial	Testing trial
Control		5.946±0.368	8.903±1.870
SCOP	1.5	10.270±1.712	7.956±0.748
HBA + SCOP	0.5	7.383±0.997	9.140±1.790
	1.0	7.736±1.191	8.381±0.750
	5.0	10.790±1.373	9.010±1.150
	25.0	9.531±1.516	7.574±1.179
PIR + SCOP	50	8.653±1.742	6.589±1.548
	100	8.282±1.664	6.047±1.171
	300	8.328±1.017	5.905±0.871

The results are expressed as mean ± S.E. n=8.

Table 2. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam (PIR) on the step-through latency of unshocked rats given PCA.

Drug	Dose	Step-through Latency (sec)	
	(mg/kg)	Training trial	Testing trial
Control		5.946±0.368	8.903±1.870
PCA	1.5	8.727±3.216	9.748±1.341
HBA + PCA	0.5	10.177±2.682	12.545±2.367
	1.0	10.290±2.892	9.708±3.050
	5.0	8.820±2.108	6.553±1.390
	25.0	11.173±2.808	9.678±1.291
PIR + PCA	50	8.207±1.846	7.864±1.257
	100	7.260±2.158	8.175±2.229
	300	8.405±2.290	13.577±4.555

The results are expressed as mean ± S.E. n=8.

Table 3. Effects of *p*-hydroxybenzyl alcohol (HBA) and piracetam (PIR) on the step-through latency of unshocked rats given APO.

Drug	Dose	Step-through Latency (sec)	
	(mg/kg)	Training trial	Testing trial
Control		5.946±0.368	8.903±1.870
APO	1.0	8.240±1.329	7.881±1.109
HBA + APO	0.5	9.019±1.210	8.240±0.503
	1.0	9.160±0.867	5.875±0.880
	5.0	9.496±1.163	6.186±1.306
	25.0	10.534±1.099	7.100±0.612
PIR + APO	50	9.973±1.932	7.684±1.549
	100	10.270±1.197	8.081±1.981
	300	9.211±0.943	7.394±1.197

The results are expressed as mean ± S.E. n=8.

Experiment 6: HBA in Combination with Various Induced Drugs in Flinch-Jump Test

As shown in Table 4, the flinch and vocalization thresholds of HBA were not different from the threshold for vehicle-treated rats. The effects of HBA in combination with SCOP, PCA, APO on the flinch-jump test were shown in Table 5, not different from the threshold for various drug-treated rats.

Discussion

Acquisition of learned responses involves modification of neurotransmission in the central nervous system. It has long been suggested that the central cholinergic system plays a major role in learning and memory in both humans and animals.

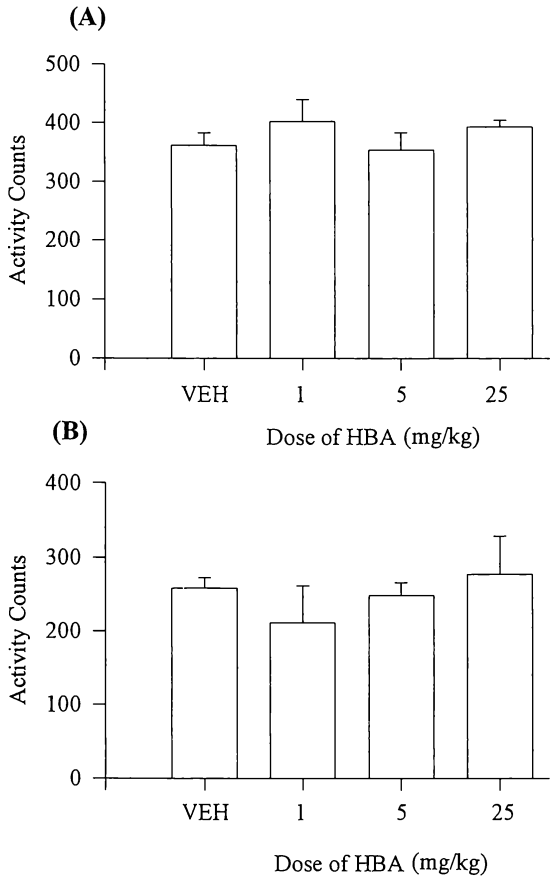

Fig 5. Effects of *p*-hydroxybenzyl alcohol (HBA) on movement activity measured (A) 1 hr and (B) 25 hr after HBA injections in rats. Values are means ± SEM for 6 rats.

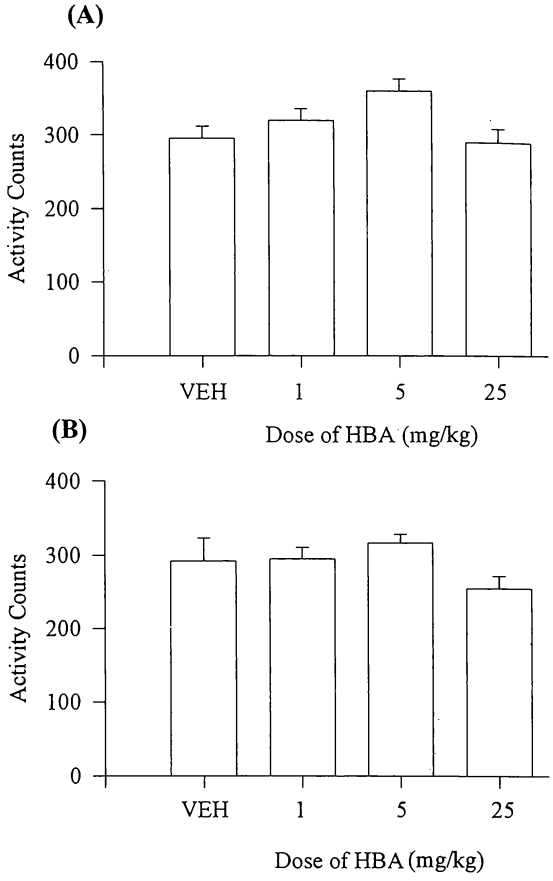

Fig 6. Effects of *p*-hydroxybenzyl alcohol (HBA) in combination with SCOP on movement activity measured (A) 1 hr and (B) 25 hr after HBA injections in rats. Values are means ± SEM for 6 rats.

Activation of this system reportedly improves learning and memory, while blockade of it impairs such processes (19). Pre-training administration of SCOP, a muscarinic receptor antagonist, at lower doses is known to block cholinergic transmission and impair acquisition in rodents (11). In this present study, SCOP at 1 mg/kg or lower dosage also significantly reduced the step-through latency, which was rectified by pre-training administration of piracetam at 100 mg/kg. Such findings are consistent with an early report (20). However, pre-training administration of HBA in various doses did not alter the deficits induced by SCOP at any dosage and was inconsistent with that of our previous study after chronic administration (5). Liu and Mori have pointed out HBA has antioxidant effect and prevents DNA degradation (21). Long-term, but not acute, protein degradation usually is found in the patients with Alzheimer's disease and caused the decline of acquisition performance (22). Furthermore, the importance of the macro-molecular synthesis in learning and memory has also been discussed (23). Therefore, the difference between acute and chronic administration of HBA on learning and memory might be related to the degradation and synthesis of protein and DNA. The detailed mechanism of HBA after chronic administration must remain to be further evaluated.

In additional to the cholinergic system, the serotonergic and the dopaminergic systems also play important roles in learning and memory. Recent studies have further revealed that these two systems exert inhibition on the cholinergic system. In anatomical studies, serotonergic neurons innervate the cholinergic neurons projecting to the basal forebrain, hippocampus and cortex (24). Dopaminergic neurons also innervate the cholinergic neurons projecting to the striatum and limbic system (25). Stimulation of the serotonergic or dopaminergic system could decrease the cholinergic activity in the above areas (25-26). In behavioral studies, serotonin or dopamine agonists could aggravate scopolamine-induced impairments in the various tasks including the inhibitory avoidance task and the radial arm maze (27-28). In this present study, PCA also significantly shortened the step-through latency. Pre-training administration of piracetam or HBA counteracted

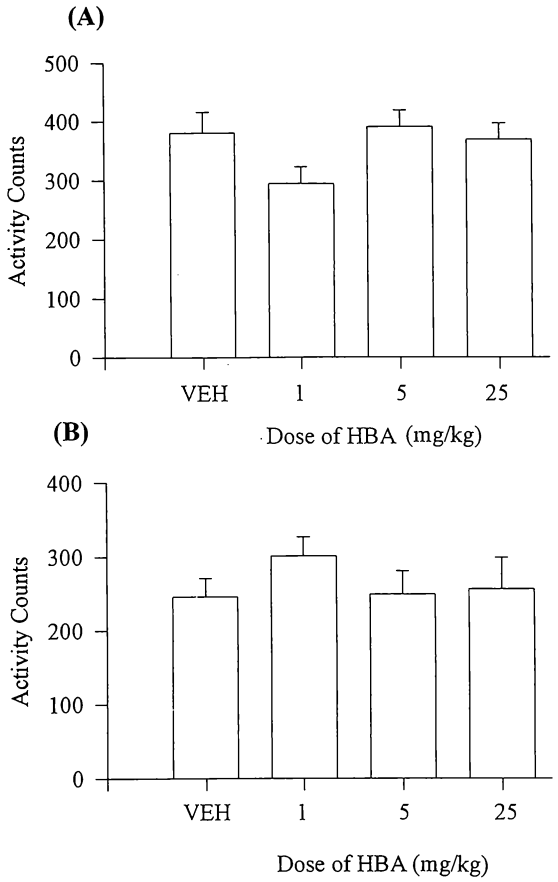

Fig 7. Effects of *p*-hydroxybenzyl alcohol (HBA) in combination with PCA on movement activity measured (A) 1 hr and (B) 25 hr after HBA injections in rats. Values are means ± SEM for 6 rats.

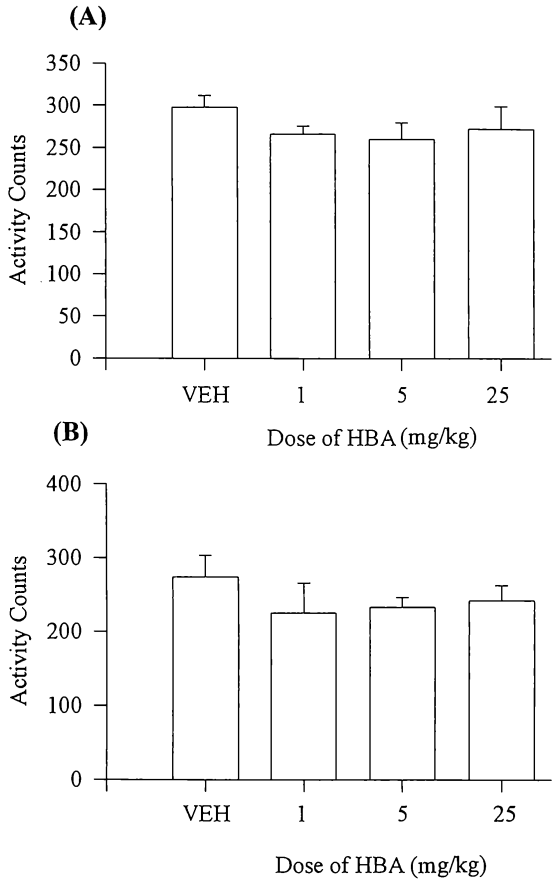

Fig 8. Effects of *p*-hydroxybenzyl alcohol (HBA) in combination with APO on movement activity measured (A) 1 hr and (B) 25 hr after HBA injections in rats. Values are means ± SEM for 6 rats.

PCA induced shortening of retention latency. In this present study, APO also significantly shortened the step-through latency of the retention trial in inhibitory avoidance task. HBA or piracetam counteracted APO induced shortening of retention latency.

The present data further support that piracetam attenuated various drug-induced impairments in the inhibitory avoidance task, which are caused not only by decrease of acetylcholine but also by altered contents of dopamine and serotonin (29). But HBA, differing from piracetam, selectively rectified the PCA-induced and the APO-induced acquisition deficits, but not the deficit induced by SCOP at any dosage. In a recent study, PCA-induced acquisition impairment in inhibitory avoidance task may be partially due to a decrease in acetylcholine release by activating the serotonergic system (30). 5-HT_2 receptor antagonist ritanserin could attenuate the PCA-induced deficits, but not the SCOP-induced deficit. However, cholinergic agonist arecoline could attenuate PCA-induced and the SCOP-induced deficit (30). Furthermore, earlier biochemical study has appeared GE extract could decrease the dopamine concentration in rat brain (31). Therefore, this improving effects of HBA are different from those of piracetam and might be related to enhance the cholinergic activity indirectly by decreasing the dopamine concentration or mediated by heterocepters on the acetylcholine terminal, but not directly by acting the postsynaptic cholinergic receptors.

The acquired behavior of animal in the inhibitory

Table 4. Effects of *p*-hydroxybenzyl alcohol (HBA) on electric foot-shock in rats.

Drug	Dose (mg/kg, PO)	Threshold (mA) Flinch	Jump / Vocalization
Control		0.75±0.03	0.88±0.01
HBA	0.5	0.78±0.02	0.86±0.01
	1.0	0.74±0.02	0.86±0.01
	5.0	0.78±0.02	0.88±0.01
	25.0	0.76±0.02	0.88±0.02

The results are expressd as mean ± S.E. n=8

Table 5. Effects of *p*-hydroxybenzyl alcohol (HBA) on electric foot-shock in various drug-treated rats.

Drug	Dose	Threshold (mA)	
	(mg/kg, PO)	Flinch	Jump / Vocalization
SCOP		0.77±0.02	0.88±0.03
HBA+SCOP	1.0	0.76±0.02	0.87±0.03
	5.0	0.78±0.04	0.89±0.03
	25.0	0.79±0.02	0.89±0.02
PCA		0.79±0.02	0.89±0.04
HBA+PCA	1.0	0.75±0.03	0.88±0.03
	5.0	0.75±0.02	0.87±0.03
	25.0	0.77±0.03	0.87±0.03
APO		0.79±0.03	0.88±0.03
HBA+APO	1.0	0.77±0.04	0.88±0.03
	5.0	0.76±0.04	0.89±0.04
	25.0	0.78±0.02	0.89±0.04

The results are expressed as mean ± S.E. n=8

avoidance task is influenced by alterations of arousal, motivation or motor activity. If animals have been administered various drugs before the training trial, the exploratory behavior, motor activity and pain sensitivity during the training trial were altered. Then, the response of animals in the inhibitory avoidance task can be indirectly altered. The behavior such as motivation and the threshold of footshock observed in the inhibitory avoidance task which received HBA or piracetam in combination with induced-deficit drugs such as SCOP, PCA and APO was grossly normal in the training or retention trial. On the other hand, the movement activities measured by open-field test were not different among the rats which received induced-deficit drugs such as SCOP, PCA and APO alone or in combination with HBA. These results revealed that the animals' behavior included motor activity, pain threshold and attention were not altered and the inhibitory avoidance performance was not influenced by non-memory behavior. Furthermore, it suggested that the attenuating effects of HBA or piracetam in various drugs-induced impairments mainly were related to memory-related process in the inhibitory avoidance task.

The results of the present study suggest that HBA, in distinction from piracetam, attenuate only PCA-induced and APO-induced impairment, but not SCOP-induced impairment in the inhibitory avoidance task. It appears that HBA acts through the decrease in dopamine concentration or other monoamine concentration and improves learning acquisition impairment. However, the further mechanisms of HBA should be studied.

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